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DROSOPHILA SELECTED FOR EXTENDED LONGEVITY ARE MORE SENSITIVE TO HEAT SHOCK

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ABSTRACT

It has been demonstrated in several animal models that a brief non-lethal application of high temperature is capable of inducing an increased longevity. It is also known that an even briefer exposure to a non-lethal elevated temperature enables some organisms to subsequently survive what would normally be a lethal exposure to high temperature. Our long-lived La strain is significantly resistant to oxidative stress due to an enhanced expression of certain antioxidant defense genes and enzyme activities. We collected survival data on 12, 463 adults of normal-lived and long-lived strains of *Drosophila melanogaster* in order to determine if animals selected for extended longevity also had an enhanced resistance to heat shock, and whether they exhibited thermotolerance as well. We find that normal-lived animals exhibit a heat-induced longevity extension but that long-lived animals already resistant to oxidative stress exhibit a heat-induced longevity shortening. The effects of temperature stress on longevity are strain dependent and are separable from thermotolerance effects. The trait of extended longevity based on an increased resistance to oxidative stress in the adult may be purchased at the price of a decreased fitness of the adult to other important environmental parameters.

INTRODUCTION

The interactions between an organism's response to stress and its life-history traits have been examined in a number of model systems by many different laboratories. The topic is interesting because it seems that such interactions can act so as to limit the potential for adaptation to changing environments. One useful approach to analyzing this topic is to examine correlated responses to selection for some important life-history trait. An examination of the comparative ability of various long-lived strains in several different species to respond to different types of stress suggests that the ability to respond to stress plays an important role in determining longevity (Johnson et al., 1995; Martin et al., 1996). However, we have shown that, in *Drosophild* at least, different long-lived strains have different patterns of resistance to various stresses (Force et al., 1995), an observation which implies that it may not be appropriate to overgeneralize the relationship between any particular stressor and longevity.

It has been demonstrated in several animal models that a brief non-lethal application of high temperature is capable of inducing an increased longevity (Lithgow et al., 1995; Khazaeli et al., 1997). It is also known that an even briefer exposure to a non-lethal elevated temperature enables some organisms to subsequently survive what would normally be a lethal exposure to high temperature (Lithgow et al., *1994,* 1995; Khazaeli et al, 1997). Such thermotolerance has been shown effective in preventing heat induced developmental defects in *Drosophild* (Petersen, 1990).

It is known that one major effect of heat stress is the heat induced expression of heat shock proteins (hsps) which are known to protect against thermal stress and act as molecular chaperones (Parsell and Lindquist, 1993). In *Drosophila,* this effect is known to be age-dependent such that old animals are less resistant to heat than young ones and have unusual patterns of hsps synthesis (Niedzwiecki et al., 1991; Fleming et al., 1988). However, it is also known that heat shocks can have other effects. They can result in an unusually high concentration of abnormal proteins which must then be degraded by the ubiquitin-dependent proteolytic system (Niedzwiecki and Fleming, 1993). Additionally, they can induce altered expression levels of molecules involved in the protection against other of stresses, and these may also contribute to the heat stress phenotype. In *Drosophila,* for example, the heat stress induced expression changes in CuZn superoxide dismutase (CuZnSOD) RNA and enzyme activity levels may play an important role in protecting the organism against any increased metabolic production of oxyradicals brought about by the elevated temperatures (Fleming et al., 1994).

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The prior studies in *Drosophila* were done using wild type or normal-lived laboratory strains. We have generated a series of strains selected for extended longevity (Luckinbill et al, 1984; Arking, 1987) and have shown that one of these strains (La) is dependent for its extended longevity on a coordinated early increase in various antioxidant protective molecules such as CuZnSOD when compared to its progenitor normal-lived (Ra) strain (Dudas et al., 1995; Arking, 1998a; Arking et al, 2000). We know that the La strain is not resistant to other stressors which appear to be important factors in other independently generated long-lived strains such as the O strains of Rose (Force et al., 1995). The observation of Khazaeli et al (1997) that heat shock increases longevity in normal-lived strains raised the question as to how widespread this phenomena is in wild type *Drosophila* strains, and whether it would also be present in strains selected for increased longevity. We thus investigated whether this phenomena would occur in both our normal-lived Ra and long-lived La strains. Those same investigators also noted that their normal-lived strain exhibited thermotolerance, and we wanted to know if such a phenomenon occurred in our two strains as well. Finally, we wanted to investigate whether the heat shock effect would be agedependent in our strains.

MATERIALS AND METHODS

Stocks: We used our normal-lived Ra and long-lived La strains which have been described previously (Arking, 1987; Buck and Arking, 1995). Breeding bottles with controlled larval density were established and mixed sex cohorts were collected from them within 24 hrs of eclosion. They were maintained in standard fly bottles on our standard sucrose-agar food (Luckinbill et al., 1984) at a density of ca. 150 flies/bottle at 25°C on a 12:12 light-dark cycle. The experimental and control flies used in all of the experiments were collected within a two-week period from the same sets of breeding parents.

Experimental flies: All experimental and control flies used in any one experiment were members of the same 48 hr cohort. Once collected, the animals were changed into new food bottles every three days. Heat shocks were administered as described at the appropriate ages. Following treatment, the number of dead animals in each bottle was counted at the time of bottle changing until all the animals in the cohort had died. All bottles comprising the experimental and control cohorts of any one experiment were manipulated as a group and treated simultaneously.

Longevity experiments: To determine whether exposure to non-lethal heat affects longevity at normal temperatures in either the Ra or La strain, we transferred 5-7 day old experimental animals of each strain into empty fly bottles containing only water saturated filter papers and heat treated them at 37°C for 100 min. Preliminary work had shown that these were non-lethal conditions for our flies (data not shown). Following the heat treatment, the animals were transferred back into fresh food bottles and maintained at 25°C as described for the remainder of their lives. Control animals were transferred into empty fly bottles containing only water saturated filter papers for the same period of time but were maintained at 25°C for their entire lifetime and were never subjected to heat treatment. A similar treatment was used for middle-aged and old animals save that they were heat treated at 27-29 days and 62-64 days of age respectively. An insufficient number of old Ra animals led to our being able to test only old La animals.

Thermotolerance experiments: To determine whether exposure to a shorter non-lethal heat treatment would lead to a subsequent protection against a later lethal heat treatment, we subjected young (7-9 day) experimental animals to a 36°C heat treatment for 70 min under conditions of saturated humidity. Two days later both the experimental and control animals were subjected to a 36°C heat treatment for 240 minutes under conditions of saturated humidity. These procedures are identical to those used by Khazaeli et al.(1997). Following the treatment, the animals were transferred back into fresh food bottles and maintained at 25°C as described above.

Data analysis: The survival data from all the cohort bottles of each experiment were pooled. Life tables were constructed as described in Chapter 2 of Arking (1998b). The *Survival* functions of SPSS for Windows v7.0 (SPSS, Inc, Chicago) were used to generate survival and mortality curves using the Kaplan-Meier protocol. The log-rank (Mantel-Cox) test was used to test longevity differences between the experimental and control strains in each experiment.

RESULTS

Complete life spans were measured on 8644 animals in the age-specific heat treatments in the Ra and La strains, and on 3819 animals in the thermotolerance experiments in the Ra and La strains. A summary of all experiments is shown in Table 1 and the survival curves for each set of control and experimental populations are shown in Figs. 1 and 2.

In the age-specific heat shock experiments, the critical question is whether the temperature treatment resulted in a significant alteration of longevity in the experimental population relative to their controls and, if so, in which direction. The existence of such changes is shown by a comparison between experimental and control cohorts of the remaining life expectancy, e_x, after the time of the heat treatment at the indicated age (9, 28, or 66 days as appropriate), and of the survival curves for the remaining life span. The mean longevity values indicate the effect of such changes on the population's life span.

There is a major difference in the response of the normal- and long-lived strains to thermal stress. The young Ra animals do display a significant increase in longevity when subjected to a non-lethal heat stress when compared to their non-treated control sibs (Table 1

Table 1. Life expectancies and mean longevities in heat treated and control populations of strains selected for different life spans.

Strain, Experiment & Cohort	*Young*				*Middle-Aged*				*Old*			
	N_9	e_9	*Mean*	*LS S.E.*	N_{28}	e_{28}	*Mean*	*LS S.E.*	N_{66}	e_{66}	*Mean*	*LS S.E.*
Ra: Heat treatment												
Control	756	8.09	36.74	0.39	522	6.34	38.71	0.29				
Experimental	1180	8.59	38.23	0.37	678	6.75	39.05	0.27			nd	
		Log Rank=17.84				*Log Rank=1.29*						
		P<0.00005				*P=0.26*						
La: Heat treatment												
Control	777	14.42	58.8	0.49	600	8.94	58.43	0.51	704	1.78	74.30	0.20
Experimental	1459	12.70	52.9	0.39	365	8.72	57.28	0.67	951	0.89	70.10	0.22
		Log Rank=57.10				*Log Rank=1.23*				*Log Rank=89.51*		
		P<0.00005				*P=0.27*				*P<0.00005*		
Ra: Thermotolerance												
Control	1396	6.54	27.91	0.28								
Experimental	1176	6.63	28.27	0.30		___				___		
		Log Rank=0.14										
		P<0.71										
La: Thermotolerance												
Control	432	12.44	45.10	0.55								
Experimental	592	11.59	44.36	0.65		___				___		
		Log Rank=0.65										
		P<0.42										

Notes: N_x, population size at day 9, 28, or 66 as indicated; e_x, remaining life expectancy on day 9, 28, or 66 as indicated; SE, standard error; Log Rank (Mantel-Cox) test=statistic and P value for the survival curve data.

and Fig, 1A). However, this response disappears in the middle age flies, there being no statistically significant difference in longevity characteristics between the Ra experimental and control populations (Table 1 and Fig. 1B). Note that there is, however, a non-significant increase in longevity values of the middle aged Ra-HX population relative to its Ra-CON population.

The young La animals also show a significant difference between experimental and control populations but it is in the opposite direction from that observed in the Ra animals. The heat treated young La animals have a significant reduction in life expectancy and mean longevity relative to their untreated controls (Table 1 and Fig. 1C. This thermal sensitivity is not observed in the middle aged La animals, although once again it must be noted that both the remaining life expectancy (e_{28}) and mean life spans are (non-significantly) lower in the experimental populations relative to the control populations (Table 1 and Fig. 1D). Finally, the old La heat-treated animals show a significant sensitivity to thermal stress relative to their untreated controls (Table 1 and Fig. 1E).

Neither the Ra nor La strain experimental populations show any significant indication of thermotolerance when compared to their respective control populations (Table 1 and Fig. 2). It must be noted, however, that the normal-lived Ra experimental animals show a (non-significant) increase in their remaining life expectancy and mean longevity relative to their controls; while the long-lived La experimental animals show a (non-significant) decrease in their remaining life expectancy and mean longevity relative to their controls.

DISCUSSION

In the normal-lived Ra strain, a 70 min heat treatment adds about 1.5 days to the mean longevity of a mixed sex young population (Table 1). This is a significant extension of longevity. In addition, there is a modest increase in the mean longevity of middle-aged Ra animals (Table 1). Although non-significant by the log-rank (Mantel-Cox) test, the less powerful Breslow and Tarone-Ware statistics indicate that the indicated difference is significant at the 0.05 level, suggesting that an increased number of animals tested might have yielded a statistically significant outcome. These observations confirm the report of Khazaeli et al. (1997) that significant heatinduced longevity extension occurs in *Drosophila* wild type strains, and may be a common response mechanism in normal-lived strains.

On the other hand, our selected long-lived La animals are very sensitive to a 70 min heat treatment and consistently show lower longevity values relative to their controls throughout their life span (Table 1). We know that the La strain is significantly resistant to oxidative stress (Force et al., 1995) due to an early and coordinate increase in the expression of their antioxidant genes, including CuZnSOD (Dudas and Arking, 1995; Arking, 1998a). Thus, selection for an increased resistance to oxidative stress does not automatically convey an increased resistance to thermal stress. Preliminary data suggests that this phenomenon is not limited to the La strain and may well be a general aspect among our

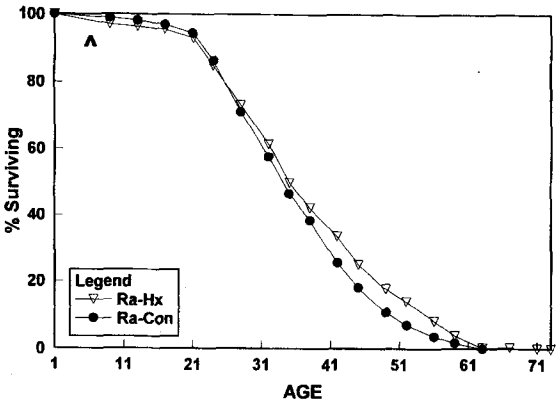

Figure 1A

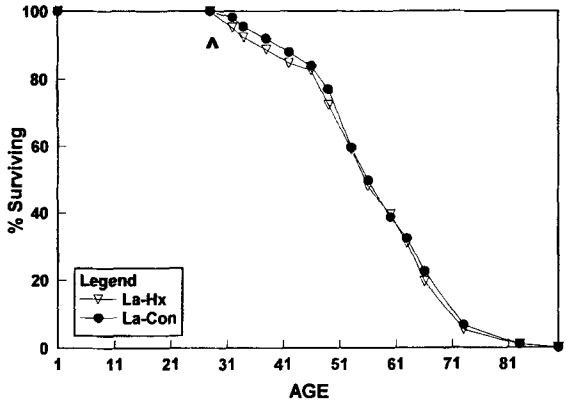

Figure 1B

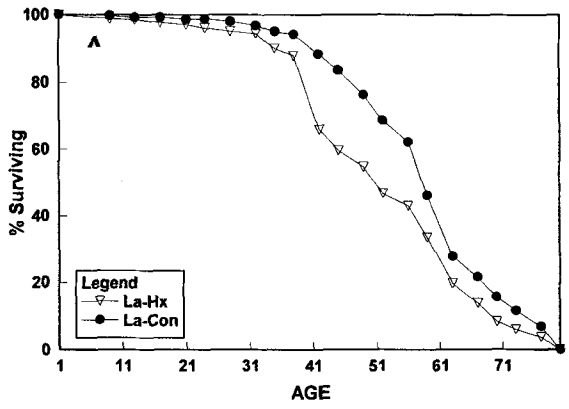

Figure 1C

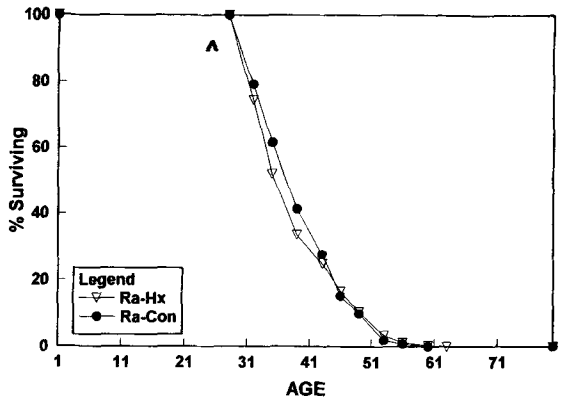

Figure 1D

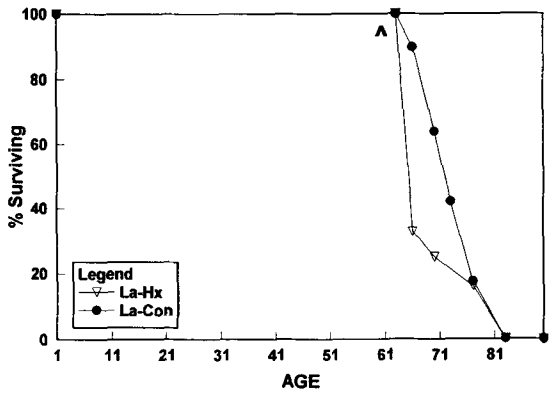

Figure 1. Survival curves of contemporaneous experimental (HX; open triangles) and control (CON; solid circles) populations of normal-lived (Ra; Figs 1A and 1B) and long-lived (La; Figs. 1C - 1E) of Drosophila melanogaster at young (5-7day) (Figs. 1A and 1C), middle-aged (27-29 days) (Figs. 1B and ID) and old (62-64) (Fig. 1E) ages. Experimental populations received a nonlethal heat stress of 100 min at 37°C and were then maintained at 25°C thereafter. Control populations received no heat stress. Arrowheads indicate the approximate time of each heat shock. See Table 1 or text for details.

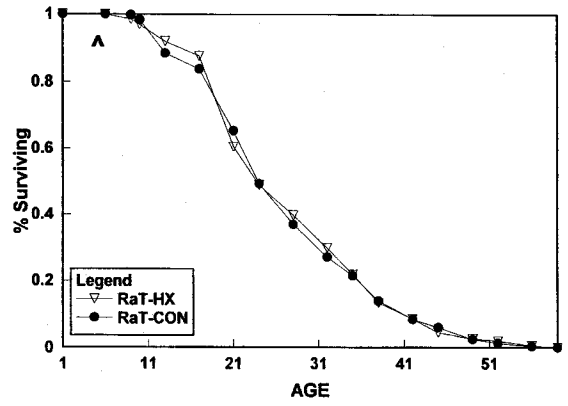

Figure 2A

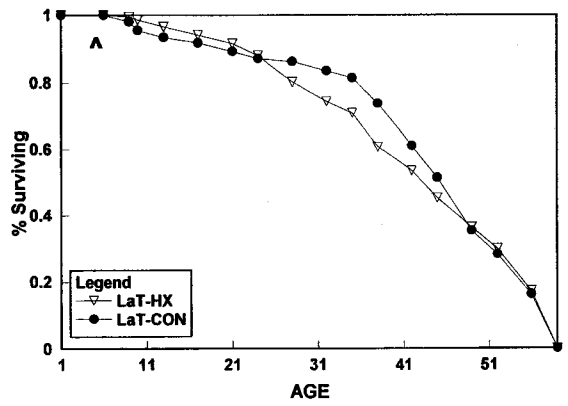

Figure 2B

Figure 2. Survival curves of contemporaneous experimental (HX; open triangles) and control (CON; solid circles) populations of normal-lived (Ra; Fig. 2A) and long-lived (La; Fig. 2B) of Drosophila melanogaster at young (5-7day) ages. Only the experimental populations received a non-lethal heat stress of 70 min at 36° C . Two days later both experimental and control populations received a lethal heat stress of 240 min at 36° C and were then maintained at 25° C thereafter. Arrowheads indicate the approximate time of each heat shock. See Table 1 or text for details.

several different long-lived strains.

In other laboratory strains, Niedzwiecki et al (1992) showed that CuznSOD gene expression is increased by a 60 min heat treatment in an age-dependent manner such that young animals show the largest increase. Since the La animals also have increased expression of CuZnSOD but are nonetheless heat sensitive, it does not seem possible that increased antioxidant levels can be a general mechanism conferring resistance to thermal stress (Fleming, 1994). Khazaeli et al. (1997) suggested that a decreased reproductive effort in heat-treated flies does not appear to be a viable explanation for the heat induced longevity extension they observed in their strains. Our data do not provide any other information on the mechanism underlying the observed longevity extension in normal-lived animals other than to specify that it is strongly strain-dependent and possibly agedependent as well. We have shown elsewhere (Arking, 1998a) that there appears to exist an inverse relationship between the levels of antioxidant defense enzymes and the levels of enzymes specific for other types of stresses (e.g., P450s). Perhaps such a relationship holds as well between the antioxidant defense enzymes and the heat shock proteins known to be involved in mitigating against thermal stress. Answering this question will be a topic of future research.

An examination of the thermotolerance data for both strains shows that a 240 min exposure to 36° shortens by ca. 30% the mean life spans of all four test populations relative to their untreated young Ra or young La control populations (Table 1). This is a lethal heat treatment. However, there is no statistically significant protective effect of an early non-lethal heat treatment on either strain's ability to survive this later 240 min lethal heat shock (Table 1). There are modest and non-significant differences in each strain's control and experimental longevity values which are consistent with the differing effects of heat shock on the Ra versus the La strains as described above. Perhaps an increase in the sizes of the populations tested or an optimization of the thermotolerance temperature protocols for these particular strains would yield significant differences in a future experiment. In any event, the data of Table 1 demonstrate that thermotolerance is separable from heat shock induced longevity alteration in the La and Ra strains.

Normal-lived animals exhibit a heat-induced longevity extension. Long-lived animals exhibit a heat-induced longevity shortening. The effects of temperature stress on longevity are strain dependent and are separable from thermotolerance effects.

Our data do suggest that long term adaptation to one set of gene-environment interactions can preclude individual adaptation to a changing environment which involves another set of geneenvironment interactions (Economos and Lints, 1986; Parsons, 1993; Partridge et al., 1995). We demonstrate elsewhere (Force et al., 1995; Buck et al., 1999) that long-lived La animals are actually less fit in certain phenotypic characteristics than are their normal-lived Ra progenitors. Given these facts, then extended longevity is not a gift. The trait of extended longevity based on an increased resistance to oxidative stress in the adult may be purchased at the price of a decreased fitness of the adult to other important environmental parameters. One reason why extended longevity does not appear to be common in the wild may be the inability of such organisms to thrive in variable environments characterized by multiple stressors.

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